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and 2. The drawings incorporated from the cited application have been amended to include new figure numbers and reference numerals which do not overlap the figure numbers and reference numerals of the present application. The text incorporated from the cited reference has been modified to reflect the change in the two added figures.

--In general the method of the present invention is for treating a medical device having at least one surface exposed to tissue and/or blood and comprises the steps of subjecting the one surface to a low temperature plasma of an appropriate chemical agent to provide a plasma deposited layer having functional groups like amine, carboxylic, or hydroxyl groups covalently bound to the surface of the device. The plasma deposited layer is then subjected to a chemical treatment with multifunctional linkers/spacers which then become covalently bound with the plasma deposit layer. A bioactive coating is then covalently bound to spacers/linkers.

More in particular, the method of the present invention as hereinafter described utilizes a plasma chamber (not shown) of the type as described in U.S. Pat. No. 5,643,580 well known to those skilled in the art and thus will not be described in detail. Typically the plasma utilized in the method of the present invention utilizes a low temperature or cold plasma produced by glow discharge. A low temperature plasma is created in an evacuated chamber refilled with a low pressure gas having a pressure on the order of 0.05 to 5 Torr and with the gas being excited by electrical energy usually in the radio frequency range. A glow discharge is created typically in the range of 2-300 watts for low power and 50-1000 watts for high power depending on the chamber volume.

The steps for the method of the present invention are shown in FIG. 9 for the treatment of a substrate 111 shown in FIG. 10 and having first and second surfaces 112 and 113. The substrate 111 is part of a medical implant or medical device that has at least one surface which is to be treated, such as one of the surfaces 112 and 113, to achieve desirable biological activities on that surface. The substrate 111 is formed of a suitable material such as a fluorinated thermoplastic or elastomer or more specifically, by way of example, PTFE. The latter material is particularly desirable where the medical implant or medical device is in the form of small-diameter vascular grafts. The substrate can also be formed of any polymer and polymer composites, metals and metal-polymer composites.

Let it be assumed that the surface 112 of the substrate 111 is to be treated in accordance with the method set forth in FIG. 9. The surface 112 is cleaned in an oxygen or air plasma as shown by step 116 in a relatively short period of time. The plasma cleaning process is an ablation process in which radiofrequency power, as for example 50-1000 watts, under a

higher pressure e.g. 0.1 to 1.0 Torr at a high flow rate, as for example of at least 50 cc. per minute gas passing through the plasma chamber. Such a cleaning process can use oxygen, hydrogen alone, a mixture of oxygen with argon or nitrogen for a period of time of up to 5 minutes. Thus, a plasma of oxygen, air, or inert gases can be utilized for plasma cleaning.

Thereafter, the surface 112 after being cleaned as shown in step 117, is functionalized by subjecting the surface 112 to a pure gas or gas mixture plasma to assist in the deposition of functional groups on the surface 112 to provide a deposited layer 118 which is covalently bound to the surface 112. Other methods which can be utilized in place of the plasma deposition step 117 include a modification by irradiation with ultraviolet or laser light in the presence of organic amine or hydrazine. The plasma deposition step 117 used to achieve activation of the surface utilizes precursor gases which can include the following inorganic and organic compounds: NH<sub>3</sub> (ammonia), N<sub>2</sub>H<sub>4</sub> (hydrazine) aliphatic amines, aliphatic alcohols, aliphatic carboxylic acids, allylamine, water vapor, allyl alcohol, vinyl alcohols, acrylic acid, methacrylic acid, vinyl acetate, saturated or unsaturated hydrocarbons and derivatives thereof. Precursors can be saturated (aliphatic amines, aliphatic alcohols, aliphatic acids) or unsaturated (allyl, vinyl and acrylated compounds). Employing unsaturated precursors or operating pulsed plasma (single mode or gradient) tend to preserve functional groups rather than form defragmentation products, having the potential of introducing a significantly higher percentage of reactive groups.

The deposition step 117 can be performed in continuous or pulsed plasma processes. The power to generate plasma can be supplied in pulsed form or can be supplied in graduated or gradient manner, with higher power being supplied initially, followed by the power being reduced or tapered towards the end of the plasma deposition process. For example, higher power or higher power on/off ratios can be utilized at the beginning of the step 117 to create more bonding sites on the surface 112 which results in stronger adherence between the substrate surface 112 and the deposited layer 118. Power is then tapered off or reduced as for example by reducing the power-on period to obtain a high percentage of functional groups on the surface 112.

The plasma deposition layer 118 created on the surface 112 has a thickness ranging from 5-1000 Å. By way of example this can be a layer derived from allylamine plasma. This plasma-assisted deposition typically is carried out at a lower power that ranges from 2-400 watts and typically from 5-300 watts depending upon the plasma chamber size, pressure and gas flow rate. This step 117 can be carried out for a period of time ranging from 30 seconds to 30 minutes while being sure that the reactive group created is preserved.

When it is desired to retain only those functional groups in the layer 118 which have established stable bonds to the substrate surface 112, as for example to a PTFE surface, an optional step 121 can be performed by rinsing or washing off loosely bound deposits with solvents or buffers. Thus, deposits which are merely adsorbed on the surface 112 are rinsed and washed off and the covalently bound deposits remain on the surface. Such a step helps to ensure that parts of the coating forming the layer 118 cannot thereafter be washed off by shear forces or ionic exchanges with blood flow passing over the surface.

Plasma-assisted deposition has been chosen because it is a clean, solvent-free process which can activate the most inert substrates like PTFE. Plasma produces high energy species, i.e., ions or radicals, from precursor gas molecules. These high energy species activate the surface 112 enabling stable bondings between the surface 112 and activated precursor gas. Allylamine has been chosen as a precursor for the plasma-assisted deposition step because it has a very low boiling point of 53°C, making it easy to introduce as a gas into the plasma chamber. By using allylamine, the desire is to have radicals created by the plasma occurring preferentially at C=C double bonds so that the free amine groups created are preserved for other reactions as hereinafter described. Also, it is believed to give a high yield of the desired primary amine group on the surface 112.

In the rinsing step 121, a solvent rinse such as dimethylsulfoxide (DMSO) is used for removing all of the allylamine deposit which has not been covalently bound to the surface 112, i.e. to remove any allylamine which has only been adsorbed on the surface. Another material such as dimethylformamide (DMF), tetrahydrofuran (THF) or dioxane can be utilized as a solvent rinse. In addition, for removing polar deposits, a buffer rinse can be utilized. As soon as the rinsing step 121 has been completed and the substrate 111 dried, wetting or surface tension measurement showed very hydrophilic PTFE (layer 118) completely wet with water. The presence of free amine groups can be visualized by tagging fluorescent probes reactive with amine groups. ATR-FTIR (attenuated total reflectance-fourier transform infrared) or ESCA (electron spectroscopy for chemical analysis) may give information about the presence of amine or nitrogen in layer 118, respectively.

Subsequently, in step 123, homo or hetero multifunctional linkers/spacers react and form stable linkages with the functional groups in layer 118 obtained by the plasma-assisted deposition process. This treatment in step 123 serves to provide linkers/spacers as represented by symbols 126 in Fig. 10 to improve accessibility of coating agents, as for example peptides and proteins, to functional groups on substrates. Vice versa, it is believed that the linkers 126

enhance the exposure of peptides and proteins to the environment. Also the linkers give peptides or proteins in the final coating more space and freedom to assume their natural conformations. As a result, the covalently bound coating agents are more likely to maintain their natural conformations and therefore their bioactivity.

By way of example, primary amine groups obtained after allylamine plasma react with succinic anhydride leading to a substrate covered by linkers 126 ended with COOH groups. Thus, the coverage with linkers 126 is less thrombogenic and more cell-friendly compared to the coverage with NH<sub>2</sub> rich layer 118. The linker/spacer attachment step 123 can also be utilized to introduce desirable functional groups which can readily react with the final coating agents. For example, COOH groups at the end of linker 126 can form stable amide linkage with NH<sub>2</sub> groups in cell-adhesion peptides and proteins, anti-inflammatory peptides, anti-thrombogenic peptides and proteins, growth factors, etc. The COOH groups can also form an ester linkage with OH groups in the anti-coagulant agent heparin. Taking the nature of the substrate, functional groups obtained after the plasma, the availability of functional groups and the size and nature of the final coating agents into consideration, the chemistry and size of the linkers may be selected.

Multifunctional linkers usually have 2-20 carbon atoms in the backbone. They can be anhydrides of dicarboxylic acids, dicarboxylic acids, diamines, diols, or amino acids. Linkers can be just one molecule, a string of several molecules, such as a string of amino acids, a string of alternate dicarboxylic acids-diamines, dicarboxylic acids-diols or anhydrides-diamines. This chemical treatment step 123 hereinbefore described can also be characterized as one that introduces other desirable functional or activating groups.

Organic solvents which are miscible with water can be used as solubility enhancers to facilitate coupling efficiency between the plasma-treated substrate and the linkers (step 123) and/or coating agents (step 128) in an aqueous medium. DMSO, DMF or dioxane can be used as such solubility enhancers. They facilitate the contact between functional groups present in molecules of different hydrophilicity or hydrophobicity. After the corresponding functional groups present in molecules of different hydrophilicity or hydrophobicity come close enough to each other, chemical reactions between them can occur. So, solubility enhancers in an aqueous solution can augment the binding reactions. The solubility enhancers may also enhance the accessibility of the linker/coating agents to the functional groups on porous surfaces.

After completion of the wet chemistry linker/spacer attachment step 123, the wetting behavior/surface tension of the resulting surface can be analyzed. Appropriate techniques,

such as ESCA, SIMS, ATR-FTIR can be used to characterize the hydrophilic surface created in step 123. Fluorescent imaging of functional groups can also be carried out.

The bioactive/biocompatible coating step 128 can be carried out to provide the final layer of coating 131 on the surface 112 of the substrate 111 (as shown in Fig. 10). In this step, the available functional groups provided by the linkers 126, are used to covalently bind molecules of a bioactive/biocompatible agent, such as a cell-adhesion peptide P15 as hereinafter described, possessing desirable properties to the substrate surface 112 to provide the final resulting coating on the surface 112 as for example a PTFE surface. Of interest are bioactive/biocompatible coatings which, among others, can reduce foreign body reactions, accelerate the functioning and integration, as well as increase the long-term patency of implants. Such coatings can include cell adhesion peptides, proteins or components of extra-cellular matrix to promote cell migration and proliferation, leading to a rapid and complete coverage of the blood-contacting surface by a natural endothelial cell lining. Coatings with growth factors such as VEGF may lead to similar results. Non-adhesive coatings with polyethylene glycol derivatives are used for biocompatible hydrophilic surfaces as separation membranes, immuno barriers or surfaces free of platelet adhesion. Also, anti-thrombogenic coatings with hirudin, hirudin analogs, reversible and irreversible thrombin inhibitor peptides, or anti-coagulant coatings with heparin are desirable to reduce or prevent thrombosis formation at the implanting site. These local anti-thrombogenic or anti-coagulant coatings are more preferable than a systemic anti-coagulant treatment. Anti-inflammatory coatings can be used because occlusions may originate at inflamed sites. Anti-proliferative coatings are another way to reduce vessel occlusions by preventing smooth muscle cell proliferation.

Chemical/biological testing such as AAA (amino acid analysis), *in vitro* cell cultures followed by SEM (scanning electron microscopy), and *in vivo* testing can be used for evaluating the coatings of the present invention.

A specific example of a coating having biological activity and medical implants having a surface carrying the same and the method incorporating the present invention may now be described as follows.

Let it be assumed that it is desired to coat long porous PTFE tubes, as for example having a length of 11 cm., which are to be utilized as medical implants and to be treated with a coating using the method of the present invention. The tubes can be prepared for treatment by mounting the same on an anodized aluminum wire frame and then inserting them in a vertical position in the upper portion of the plasma chamber being utilized. The tubes are then cleaned

in an air plasma by operating the plasma chamber at 0.3 Torr at 50 watts for 3 minutes. After the plasma cleaning operation has been performed, the chamber is flushed with allylamine gas at 0.2 Torr for 10 minutes. Allylamine plasma is then created at 0.2 Torr at 15 watts for 30 minutes. Radiofrequency power is turned off and allylamine is permitted to flow at 0.2 Torr for 2 minutes. The allylamine flow after plasma treatment is provided to react with any free radicals on the PTFE. The allylamine flow is then terminated and a vacuum is maintained in the chamber for 15 minutes. Thereafter, the pressure in the plasma chamber is increased to atmospheric pressure. The tubes being treated are then removed from the chamber and transferred to clean glass rods. The tubes are then submerged and rinsed in an appropriate volume of DMSO. The samples are then removed from the DMSO rinse and washed with deionized (DI) water and optionally ultrasonically at room temperature for 3 minutes.

In the covalent linker attachment step 123, a 1 M (one molar) succinic anhydride solution is prepared using DMSO and placed in a covered glass tray container. The plasma treated and optionally rinsed tubes are then submerged in the succinic anhydride solution in the glass tray container and subjected to an ultrasonic mix at 50°C in order to bring the succinic anhydride into close proximity to the free amine groups on the PTFE surface. A one molar (1M) Na<sub>2</sub>HPO<sub>4</sub> solution in DI water is used to adjust the pH between 6 to 9, preferentially pH 8. A higher pH results in a faster reaction. This reaction between the free amine groups and the succinic anhydride can be carried out between room temperature and 80°C and preferentially between 20-50°C.

After this has been accomplished, the tubes are removed and rinsed with DI water optionally utilizing ultrasound. The tubes are then dried with nitrogen.

Let it be assumed that a peptide coating is desired to be applied to the surface thus far created. Solubility enhancers such as DMSO and DMF can be added between 0-50 volume/volume v/v %, preferentially 10-30%. A 90 mL. DI water/DMSO solution is prepared by taking 70 mL. of DI water and mixing the same in a glass container with 20 mL. of DMSO. The dried tubes are then placed in the DMSO solution and ultrasonically mixed for a period of 1 minute.

Freshly prepared EDC [N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride] (Fluka) solution in 5 ml DI water is poured over the tubes submerged in water/DMSO to activate COOH groups on the PTFE surface. After 0.5-3 min., P15 ((H-Gly-Thr-Pro-Gly-Pro-Gln-Gly-Ile-Ala-Gly-Gln-Arg-Gly-Val-Val-OH; SEQ ID NO: 1) acetate salt, GLP grade peptide) solution in 5 ml DI water is added. For hydrophobic peptides, the peptides may be dissolved in an organic

solvent miscible with water (DMSO, DMF or dioxane). EDC and P15 amounts are based on the following final concentrations: 0.02 M EDC to be used and 0.0002 M P15 in the final reaction volume, i.e. 100 x molar excess of EDC to P15. The reaction at room temperature is carried out between 1-16 hours, preferentially 2-8 hours. The tubes are then rinsed several times with deionized water with an optional one minute ultrasonic treatment. The tubes are then dried with nitrogen gas. The tubes are then inverted to bring the coated side to the inside. Amino acid analysis revealed that up to 1.5 nmol P15/cm<sup>2</sup> was bound to the PTFE surface.

From the foregoing it can be seen that there has been provided a coating which has biological activities which can be utilized on surfaces of medical implants and devices and a method for accomplishing the same. The coating and method can be utilized on many different types of devices which are intended to be implanted in the human body or in other words to remain in the human body for a period of time. Such devices include stents and grafts placed in various vessels of the human body. Other medical devices such as heart valves, defibrillators and the like have surfaces which are candidates for the coating and method of the present invention. The coating and method is particularly advantageous for use on surfaces which heretofore have been difficult to obtain cell growth on, as for example PTFE and ePTFE. By utilizing the coating and method of the present invention, it has been found that cell growth has been greatly enhanced, making possible long term implantation of said devices in the human body.--

Please insert the paper copy of the Sequence Listing filed herewith, beginning on a separate page following the claims.

In the Drawings:

Enclosed herewith, for approval by the Examiner and Draftsman, are new Figs. 9 and 10 corresponding to Figs. 1 and 2, respectively of the above-mentioned U.S. Application Serial No. 09/385,692 which is specifically referred to and thereby incorporated into the present application on page 7, lines 31-35.

In the Claims:

Please cancel claims 1-16 without prejudice, and add new claims 17-19 as follows: